Published in final edited form as:

Hum Genet. 2016 October ; 135(10): 1175–1179. doi:10.1007/s00439-016-1706-2.

Immunoglobulin G genotypes and the risk of schizophrenia

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Abstract

Genes of the immune system are relevant to the etiology of schizophrenia. However, to our knowledge, no large-scale studies, using molecular methods, have been undertaken to investigate the role of highly polymorphic immunoglobulin GM (γ marker) genes in this disorder. In this investigation, we aimed to determine whether particular GM genotypes were associated with susceptibility to schizophrenia. Using a matched case–control study design, we analyzed DNA samples from 798 subjects—398 patients with schizophrenia and 400 controls—obtained from the U.S. National Institute of Mental Health Repository. GM alleles were determined by the TaqMan® genotyping assay. The GM 3/3; 23−/23− genotype was highly significantly associated with susceptibility to schizophrenia ($p = 0.0002$). Subjects with this genotype were over three times (OR 3.4; 95 % CI 1.7–6.7) as likely to develop schizophrenia as those without this genotype. Our results show that immunoglobulin GM genes are risk factors for the development of schizophrenia. Since GM alleles have been implicated in gluten sensitivity and in immunity to neurotropic viruses associated with cognitive impairment, the results presented here may help unify these two disparate areas of pathology affected in this disorder.

Introduction

Schizophrenia is a highly heritable neurodevelopmental disorder characterized by disturbances in thought and perception. Genome wide association studies (GWAS) have identified numerous risk genes, but most of the heritability of schizophrenia remains unexplained, suggesting the involvement of additional genes in the etiology of this devastating disorder. Immune dysregulation has been postulated to play an important role in triggering this disease, and numerous studies showing its association with HLA, a major gene complex of the immune system, support this contention. In fact, one of the strongest associations reported in the largest ever GWAS of schizophrenia was with the genes in the HLA complex (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014), which, at least in part, may be due to the excessive complement activity in this disorder (Sekar et al. 2016).

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

GWAS in general (including the one mentioned above), to our knowledge, do not evaluate immunoglobulin GM (γ marker) allotypes, because these determinants are not included in the commonly employed genotyping platforms. Immunoglobulin heavy chain G1 (*IGHG1*), *IGHG2*, and *IGHG3* genes (chromosome 14q32), which encode GM allotypes, are highly homologous, (Lefranc and Lefranc 2012) and apparently not amenable to high throughput genotyping technology; this attribute might have contributed to their exclusion from the genotyping panels. Furthermore, since GM allotypes were not typed in the HapMap project, they cannot be imputed. Even in the 1000 Genomes project, to our knowledge, the coverage of this region is very low. Therefore, a candidate gene approach is necessary for evaluating the role of GM genes in the etiology of schizophrenia.

There is strong rationale for the involvement of GM allotypes in the pathogenesis of schizophrenia (Pandey 2014). These genes have been shown to be associated with susceptibility to many diseases, including neurological diseases. They are also associated with immunity to several infectious pathogens. Especially relevant to schizophrenia, GM alleles contribute to immunity to neurotropic viruses cytomegalovirus (CMV) and herpes simplex virus type 1 (HSV1) (Pandey et al. 2014; Moraru et al. 2015). Exposure to these viruses is associated with cognitive impairment in schizophrenia and other neurodevelopmental disorders (Shirts et al. 2008; Yolken et al. 2011; Prasad et al. 2012; Nimgaonkar and Yolken 2012; Thomas et al. 2013). Additional rationale for the GM gene involvement in schizophrenia is provided by their association with immune responsiveness to gliadin (Weiss et al. 1983). Gluten sensitivity, characterized by the presence of antibodies to gliadin, is significantly associated with schizophrenia and a variety of other neurological disorders (Porcelli et al. 2014; Okusaga et al. 2013; Jackson et al. 2012).

In this study, using a candidate gene approach, we aimed to determine whether any GM genotypes were associated with susceptibility to schizophrenia.

Subjects and methods

Study design and samples

A matched case–control design was used. DNA samples from 398 white patients with schizophrenia and 400 controls were obtained from the National Institute of Mental Health Repository. Controls were matched to case patients by ethnicity and age (within 5 years). Specimens from two patients were not available. The demographic details of the study cohorts are given in Table 1.

GM genotyping

IgG1 markers GM 3 and 17 (arginine to lysine) were determined by a pre-designed TaqMan® genotyping assay from Applied Biosystems Inc. (Foster City, CA), employing the following primers and probes:

Forward primer: 5′ CCCAGACCTACATCTGCAACGTGA-3′.

Reverse primer: 5′ CTGCCCTGGACTGGGACTGC AT-3′.

Reporter 1 (GM 17-specific): VIC-CTCTCACCAACTTTCTTGT-NFQ.

Reporter 2 (GM 3-specific): FAM-CTCTCACCAACTCTCTTGT-NFQ.

IgG2 markers GM 23− and 23+ (valine to methionine) were determined by a TaqMan® genotyping assay from Applied Biosystems Inc. employing the following primers and probes:

Forward primer: 5′ CCCGAGGTCCAGTTCAACT-3′.

Reverse primer: 5′ CGTGGCTTTGTCTTGGCATTATG-3′.

Reporter 1 (GM 23−specific): VIC-CACCTCCACGCCGTC-NFQ.

Reporter 2 (GM 23+specific): FAM- CACCTCCATGCCGTC –NFQ.

For technical reasons, five control subjects could not be genotyped. One sample (from a patient) with a very unusual genotype (GM 17/17; 23+/23+) was excluded from the analyses. Thus, statistical analyses involved GM genotype data from 397 patients with schizophrenia and 395 control subjects.

Statistical analysis

A global Chi-square test (8 × 2) was conducted to determine whether or not there was a significant difference in the distribution of GM genotypes between patients and controls. Contingency (2 × 2) Chi-square tests were conducted to determine whether any of the genotypes was individually associated with schizophrenia: 17/17; 23−/− vs. the rest; 17/17; 23+/− vs. the rest; 3/17; 23−/− vs. the rest; 3/17; 23+/− vs. the rest; 3/17; 23+/+ vs. the rest; 3/3; 23−/− vs. the rest; 3/3; 23+/− vs. the rest; 3/3; 23+/+ vs. the rest.

Results

GM genotype frequencies at both loci were in Hardy–Weinberg equilibrium in controls ($p > 0.05$). In patients, however, there was a significant departure from the equilibrium ($p < 0.05$). The distribution of eight GM genotypes in patients with schizophrenia and controls is given in Table 2. A global Chi-square test showed a significant difference in the distribution of the eight GM genotypes between patients and controls (Chi-square = 17.4; *d.f.* = 7; $p = 0.015$). Examination of the individual genotype frequencies showed that only the GM 3/3; 23−/23− genotype was significantly associated with susceptibility to schizophrenia (Chi-square = 13.2; *d.f.* = 1; $p = 0.0002$; Table 3). This association would remain highly significant even after the most conservative correction for multiple testing ($p = 0.0002 \times 8 = 0.0016$). Subjects with the GM 3/3; 23−/23− genotype were over three times (OR 3.4; 95 % CI 1.7–6.7) as likely to develop schizophrenia as those without this genotype.

Discussion

The results presented here show a distinct association between the GM 3/3; 23−/23− genotype and susceptibility to schizophrenia. The risk of schizophrenia was over three-fold higher in subjects with the GM 3/3; 23−/23− genotype, compared to those who lacked this genotype. The GM 23 (*IGHG2*) allele is almost always associated with GM 3 (*IGHG1*) in populations of European descent. However, the GM 3 allele can be expressed with or without GM 23, giving rise to two haplotypes—GM 3, 23+ and GM 3, 23−. The frequency

of the GM 3 23− haplotype is lower (24 %) than that of the GM 3, 23+ haplotype (43 %) (Oxelius and Pandey 2013). There are at least two potential mechanisms through which GM alleles could be associated with susceptibility to schizophrenia. The GM 3/3; 23−/23− genotype could itself modulate the risk of schizophrenia, possibly through its effect on immunity to environmental/viral triggers that may be relevant to the etiopathogenesis of this disorder. Alternatively, there may be another locus for susceptibility to schizophrenia on chromosome 14, distinct from GM, whose alleles are in significant linkage disequilibrium with those of the GM loci. This putative linkage disequilibrium could give rise to the associations observed.

The most relevant among the environmental triggers of schizophrenia, immunity to which is influenced by GM alleles, is gliadin. As mentioned before, increasing evidence implicates gluten sensitivity in the etiopathogenesis of schizophrenia. The GM 23 allotype—expressed on IgG2 antibodies and encoded by the *IGHG2* gene—has been shown to be associated with antibody responsiveness to gliadin (Weiss et al. 1983). The putative mechanisms underlying the association of neurological symptoms with gluten sensitivity are not completely understood, but the results from several studies suggest an immunological basis for this relationship (Khandaker and Dantzer 2016; Hadjivassiliou et al. 2002). Molecular mimicry —immune response against antigens shared by the host and the environmental trigger (gliadin)—has been postulated as a possible mediator of this association. Convincing evidence has been presented for immune cross reactivity between gliadin and synapsin 1, a cytosolic phosphoprotein found in most neurons of the central and peripheral nervous systems (Alaedini et al. 2007). Synapsin is involved in central nervous system neurotransmitter release and has been implicated in the pathophysiology of schizophrenia and bipolar disorder (Evergren et al. 2007; Vawter et al. 2002). Thus, to gain a better understanding of the relationship between the GM alleles and susceptibility to schizophrenia, large-scale studies to determine the possible contribution of these genes to the magnitude of humoral immunity to neuronal synapsin are warranted.

The most relevant among the infectious pathogens implicated in schizophrenia, immunity to which is influenced by GM alleles, are the neurotropic viruses CMV and HSV1. Increasing evidence implicates these viruses in the cognitive impairment of patients with schizophrenia. We have recently shown that the GM 3 allele is significantly associated with antibody responsiveness to CMV glycoprotein B, which is required for viral infectivity and is a major component of the viral envelope (Pandey et al. 2014). This allele is also involved in immunity to HSV1: it epistatically interacts with the Fcγ receptor IIIa (FcγRIIIa) alleles expressed on natural killer cells and contributes to an asymptomatic course of HSV-1 infection (Moraru et al. 2015). Furthermore, GM 3 and FcγRIIIa-valine alleles jointly contribute to the magnitude of antibody-dependent cellular cytotoxicity of HSV1-infected cells.

Additional mechanisms of GM gene involvement in the pathogenesis of schizophrenia could involve their modulating influence on viral immunoevasion strategies. Both CMV and HSV1 have evolved highly sophisticated strategies for decreasing the efficacy of the host immune response and interfering with viral clearance. We, and others, have shown that GM alleles modulate these immunoevasion strategies, and thus are excellent candidates to be effect

modifiers of the association between the exposure to neurotropic virus and cognitive impairment in schizophrenia (Atherton et al. 2000; Namboodiri and Pandey 2011; Pandey et al. 2015).

The current study involved white (of European descent) subjects only. Expression of certain GM alleles is ethnically/geographically restricted. Linkage disequilibrium between particular GM alleles within an ethnic group is almost absolute, and the determinants are transmitted as a group—haplotypes. Every major geographic population group (e.g., Europeans, Africans, etc.) is characterized by a unique array of GM haplotypes (Steinberg and Cook 1981). Therefore, to gain a thorough understanding of the role of the GM gene complex in the biology of schizophrenia, large multicenter, multiethnic studies are needed to address many outstanding questions, such as (1) do GM alleles contribute to immunity to *Toxoplasma gondii*, which has been implicated in schizophrenia? (2) Do GM alleles epistatically interact with certain HLA alleles and enhance the risk of schizophrenia? (3) Do GM alleles jointly with HLA alleles contribute to the magnitude of humoral immunity to gliadin? This appears to be the case in mice (Kagnoff 1982). (4) The C1q complex, which triggers the complement cascade, has been shown to bind differentially to the IgG antibodies expressing different GM alleles (Brüggemann et al. 1987). In view of the recent findings concerning the complement activity in schizophrenia (Sekar et al. 2016), it would be of interest to determine whether allotypically disparate anti-synapsin IgG antibodies have differential affinity to the complement components.

To our knowledge, this is the first report implicating GM genes in susceptibility to schizophrenia. It needs to be replicated in an independent study population. It is hoped that the results presented here would inspire further studies to investigate the role of (hitherto understudied) the GM gene complex in the immunobiology of schizophrenia.

Acknowledgments

We thank the National Institute of Mental Health Repository and its personnel for providing the DNA samples. We are highly grateful to the investigators who provided the specimens to the Repository. This work was supported in part by the NIH Grant MH104613 and by the National Research Foundation of Korea Grant NRF-2014S1A2A2028559.

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Table 1

Demographic details of the study cohorts

	N	**Age Mean ± SD**	**Gender Male/female**
Schizophrenia	398	36.8 ± 12.9	295/103
Controls	400	37.1 ± 12.6	200/200

Table 2

Distribution of GM genotypes in patients with schizophrenia and matched controls

GM genotypes	Schizophrenia *N* (%)	Controls *N* (%)
17/17; 23−/−	42 (10.6)	39 (9.9)
17/17; 23+/−	15 (3.8)	8 (2.0)
3/17; 23−/−	59 (14.8)	58 (14.7)
3/17; 23+/−	85 (21.4)	100 (25.3)
3/17; 23+/+	6 (1.5)	7 (1.8)
3/3; 23−/−	35 (8.8)	11 (2.8)
3/3; 23+/−	75 (18.8)	90 (22.8)
3/3; 23+/+	80 (20.1)	82 (20.8)

Chi-square (8 × 2) = 17.4; *d.f.* = 7; $p = 0.015$

Table 3

Number of schizophrenia patients and controls stratified by their GM 3/3; 23−/23− genotype status

GM genotypes	Cases *N* (%)	Controls *N* (%)
GM 3/3; 23−/23−	35 (8.8)	11 (2.8)
Non-GM 3/3; 23−/23−	362 (91.2)	384 (97.2)

Chi-square (2 × 2) = 13.2; *d.f.* = 1; $p = 0.0002$

OR 3.4; 95 % CI 1.7–6.7